

Hematology of Normal and Melanomatous Fishes: A Preliminary Report¹

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(Plate I)

EXTERNAL hemorrhages are often seen in some platyfish-swordtail hybrids suffering from melanomas. In the terminal stages of the black pigment cell disease, the tumorous fish are severely cachectic. It was thought that these externally visible conditions might be reflected in the blood of the tumorous animal when compared with that of a normal one. The present paper presents the results of examination of the blood of normal platyfish *Xiphophorus (Platypoecilus) maculatus*, normal swordtails *Xiphophorus helleri* and their normal and melanomatous hybrids.

MATERIAL AND METHODS

Blood from the following groups of adult fishes was examined:

1. Pure, laboratory-reared platyfish, descendants of fish taken from the Río Coatzacoalcos in Mexico.
2. Pure, laboratory-bred swordtails, descendants of fish taken from the same river system.
3. Hybrids of the group designated h68. Some of these fish had melanomas, whereas others were normally pigmented (Berg & Gordon, 1953). These were bred from a first generation melanomatous hybrid female and a male swordtail.
4. Hybrids of the strain designated 287. All members of this group were melanomatous.

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They were the first generation hybrids between a normal, spotted-belly platyfish and an albino swordtail.

The coagulation time of the blood of these animals is so short that great difficulty was at first encountered in making usable smears. This was true of both the normal and the melanomatous animals. After trying several methods of drawing the blood, the following was finally decided upon as providing the most consistent and useful results. The fish was grasped with a wet pad in such a manner as to leave the anterior portion of the ventral surface exposed. This surface was flushed with a solution of heparin in 0.6% saline containing approximately 0.5 mg of heparin per ml. The needle was applied to the fish between the gill covers, through the isthmus, and the heart was punctured. One or more drops of blood were obtained in this manner.

Blood smears were made on clean, greaseless slides in the usual manner. The smears, while still moist, were fixed by being held about 1 cm over the surface of a commercial 40% formaldehyde solution for 40 seconds, or by immersing them for 5 minutes in a saturated aqueous solution of mercuric chloride. It was found that fixing the smears while still wet eliminated much of the cellular distortion usually obtained in preparations which had been permitted to dry prior to fixation.

The blood smears which had been fixed over formaldehyde were held at least 24 hours before staining. Those fixed in the mercuric chloride solution were rinsed in water and in 70% alcohol and were stained immediately.

Staining was accomplished either by the buffered Giemsa method (Lillie, 1948) or by a modification of the May-Grunwald-Giemsa method (Bessis, 1948). The Horecker Lillie buffers were employed (Lillie, 1948): pH 6.4 for

mercuric chloride-fixed material, pH 5.7 for formaldehyde-fixed preparations.

Attempts to make counts of the total number of cells in a given volume of blood were unsuccessful, since the blood invariably clotted in the pipettes before the diluting fluid could be added.

Differential leucocyte counts were made, between 300 and 500 white blood cells being counted on each smear.

DESCRIPTION OF THE BLOOD

The blood of all the fishes used in this study contained lymphocytes, monocytes, eosinophilic granulocytes, thrombocytes and a few unclassified cells.

The lymphocytes are small round cells having an average diameter of 7.1 microns with a range of 6.0 to 8.0. The nucleus is usually round and is violet in color when stained with May-Grunwald-Giemsa. The lymphocyte nucleus is rather large in comparison with the total size of the cell, having an average diameter of 3.9 microns with a range of 3.0 to 4.5. The cytoplasm is clear blue, occasionally vacuolated, but containing granules only very rarely. Some of the lymphocytes appear to have pseudopodial processes of varying length.

The monocytes of these fishes are the largest of the formed elements of the blood, having an average diameter of 16.8 microns and ranging from 13.5 to 18.2. They are more or less round with considerable irregularity in contour. Their nuclei average 7.2 microns ranging from 6.8 to 8.0 in greatest dimension. The cytoplasm is pale blue in color when stained with May-Grunwald-Giemsa, and it contains scattered deep blue granules as well as an occasional vacuole. The nuclei are purple in color and are frequently indented or horseshoe shaped.

The granulocytes are almost all of the eosinophilic variety. These cells average 10.1 microns ranging from 8.7 to 11.8 in diameter and are generally round in shape. Their nuclei average 6.6 microns ranging from 5.9 to 7.8 in greatest dimension and are frequently deeply indented. No truly filamented nuclei are seen in our preparations. The cytoplasm is blue with the May-Grunwald-Giemsa stain, and contains numerous pink to red granules. These are not concentrated in any particular part of the cell, but are scattered throughout the cytoplasm.

The thrombocytes are round or somewhat spindle-shaped elements which vary considerably in size, but which are all smaller than the lymphocytes. They were frequently found massed together in groups of three to ten cells. With the May-Grunwald-Giemsa stain, the cytoplasm appeared blue, the nucleus being a much deeper, more intense blue.

In the blood of one h68, normally pigmented hybrid, a large number of cells containing long, rod-like, strongly basophilic bodies were found. The cells appeared to be essentially identical with normal lymphocytes or perhaps monocytes. The inclusions appear very much similar to microorganisms found in the lymphocytes of *Hippocampus* after injection of the bacteria into the abdominal cavity (Sabrazès & Muratet, 1901). They may represent *Bartonella* bodies.

A few blood cells contain numerous deep blue granules of small size. Whether or not these are true basophilic granulocytes is not clear. One cell contains large eosinophilic globules.

The erythrocytes of the fishes average 9.5 microns in long diameter and 6.7 microns in short diameter. Their nuclei were also elliptical in shape, averaging 4.1 microns in long diameter. With the May-Grunwald-Giemsa stain, the cytoplasm of most of the erythrocytes is orange to pink in color, the nucleus deep purple and reticulated. Some of the erythrocytes have a distinctly basophilic cytoplasm, being blue or blue-gray in color. This matter will be further discussed below.

Differential counts of the leucocytes of the various animals used were made. Inasmuch as the thrombocytes are frequently found in clumps containing many cells, they are not included in the results. The relative frequency of lymphocytes, monocytes and eosinophilic granulocytes in each animal's blood is shown in Table 1. It may be seen that there is no statistically reliable difference between the differential leucocyte counts of the various groups of animals. In addition, there is no correlation between the severity of the melanoma in a fish and its differential blood cell count.

With regard to the basophilic erythrocytes mentioned above, counts were made to determine their frequency in the blood of normally and abnormally pigmented fish of the strain h68. It was found that approximately 6% of the erythrocytes in the blood of each group were basophilic.

DISCUSSION

Our observations are essentially in agreement with descriptions of the blood cell types of some other teleost fishes which have been made by Drzewina (1909, 1911), Lanine (1912), Sabrazès & Muratet (1901), Kawamoto (1930) and Catton (1951). The blood of fishes suffering from infections and from deficiency diseases has been discussed by Katz (1950) and by Wolf (1951).

No differences were found between the blood of the normal and melanomatous fishes despite the fact that some of the latter were moribund.

TABLE 1. DIFFERENTIAL BLOOD CELL COUNTS IN XIPHOPHORIN FISHES. EACH COUNT IN PER CENT IS BASED ON AT LEAST 300 WHITE BLOOD CELLS

| Type and Number of Animal | Lymphocytes | Monocytes | Granulocytes |
|----------------------------|-------------|-----------|--------------|
| Platyfish | | | |
| 1 | 82 | 15 | 3 |
| 2 | 85 | 13 | 2 |
| 3 | 83 | 13 | 4 |
| 4 | 85 | 12 | 3 |
| 5 | 84 | 11 | 5 |
| 6 | 83 | 13 | 4 |
| Mean | 83.7±0.7 | 12.8±0.7 | 3.5±0.4 |
| Swordtail | | | |
| 1 | 84 | 11 | 5 |
| 2 | 82 | 14 | 4 |
| 3 | 83 | 12 | 5 |
| 4 | 83 | 14 | 3 |
| Mean | 83.0±0.3 | 12.8±1.2 | 4.3±0.2 |
| Normal Hybrids (h68) | | | |
| 1 | 84 | 13 | 3 |
| 2 | 87 | 11 | 2 |
| 3 | 85 | 11 | 4 |
| 4 | 86 | 12 | 2 |
| 5 | 82 | 14 | 4 |
| 6 | 88 | 10 | 2 |
| 7 | 89 | 9 | 2 |
| 8 | 88 | 9 | 3 |
| 9 | 83 | 14 | 3 |
| Mean | 85.8±1.9 | 11.4±1.3 | 2.8±0.1 |
| Melanomatous Hybrids (h68) | | | |
| 22 | 66 | 29 | 5 |
| 24 | 84 | 11 | 5 |
| 25 | 80 | 14 | 6 |
| 27 | 85 | 12 | 3 |
| 28 | 75 | 20 | 5 |
| 30 | 79 | 17 | 4 |
| 31 | 82 | 13 | 5 |
| 33 | 80 | 16 | 4 |
| 35 | 81 | 14 | 5 |
| 36 | 80 | 12 | 8 |
| 37 | 83 | 13 | 4 |
| 38 | 82 | 11 | 7 |
| Mean | 79.8±6.8 | 15.2±7.4 | 5.1±0.5 |
| Melanomatous Hybrids (287) | | | |
| 1 | 85 | 12 | 3 |
| 2 | 82 | 13 | 5 |
| 3 | 81 | 14 | 5 |
| 4 | 84 | 14 | 2 |
| 5 | 83 | 13 | 4 |
| 6 | 80 | 16 | 4 |
| Mean | 82.5±1.4 | 13.7±0.8 | 3.8±0.6 |

The melanomas are highly vascular and are frequently naked, the epithelium having degenerated and sloughed off. Hemorrhagic spots of blood are occasionally seen on the surfaces of the melanotic tumors, according to Reed & Gordon (1931) and Gordon & Lansing (1943). Gordon & Smith (1938) have reported that the epidermal cells which usually cover the entire body often degenerate around the melanomas. The pad used for holding the fish was frequently spotted with blood in the area which had been in contact with the tumor. For these reasons it was thought that blood differences usually associated with hemorrhage might be detected. They were not found by the method we used. Some cytochemical methods were tried later by Jakowska, Nigrelli & Gordon (1951) on similar specimens but no apparent differences were found.

SUMMARY

1. The melanomas in fishes are highly vascularized and are often hemorrhagic. It was thought these conditions would be reflected in the blood of diseased fish when compared with the normal ones.
2. The blood cells of *Xiphophorus (Platy-poecilus) maculatus* and *Xiphophorus helleri* and of their normal and melanomatous hybrids are described.
3. Lymphocytes, monocytes, eosinophilic granulocytes, thrombocytes and erythrocytes are regularly found in the blood of all the groups studied.
4. Differential leucocyte counts for the various groups are presented.
5. No differences between the normal and melanomatous hybrids were found by the methods used.

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NOTE: The following paper appeared as this manuscript was passing through proofreading:

DOMBROWSKI, HEINZ

1953. Untersuchungen über das Blut des Karpfens (*Cyprinus carpio* L.) und einiger anderer Süßwasserfischarten. *Biol. Zentralblatt* 72 (3/4): 182-195.

EXPLANATION OF THE PLATE

PLATE I

- FIG. 1. Lymphocyte.
 FIG. 2. Monocyte.
 FIG. 3. Eosinophilic granulocyte.
 FIG. 4. Cell containing bodies suggestive of bacteria.
 FIG. 5. Cell containing eosinophilic globules.
 FIG. 6. Cell containing basophilic granulation.
 FIG. 7. Thrombocyte.
 FIG. 8. Erythrocyte.